PRESCRIBED FORMAT FOR SUBMISSION OF PROPOSAL FOR TOPIC APPROVAL IN THE FACULTY OF SCIENCE AND TECHNOLOGY

PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE PH. D DEGREE IN THE SUBJECT OF PHARMACEUTICAL CHEMISTRY.

Title of the topic	:	Design, synthesis and pharmacological evaluation of epidermal growth factor receptor inhibitors as anticancer agents
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OUTLINE OF RESEARCH PROJECT FOR APPROVAL OF REGISTRATION FOR DEGREE OF PH. D. (TECH.) IN PHARMACEUTICAL CHEMISTRY OF UNIVERSITY OF MUMBAI

TITLE: Design, synthesis and pharmacological evaluation of epidermal growth factor receptor inhibitors as anticancer agents.

AIMS OF STUDY:

- To carry out structure based design for series based on heterocyclic and chalcone scaffolds as epidermal growth factor receptor (EGFR) inhibitors and synthesize the computationally favorable analogues.
- To screen the synthesized compounds for anti-cancer activity.

OBJECTIVES OF STUDY:

- To design ligands based on heterocyclic scaffolds such as 1,3,4-thiadiazole, indole, quinazoline and chalcone moiety.
- To carry out *in silico* ADME studies of the designed analogues on heterocyclic series such as 1, 3, 4-Thiadiazole, Indole, Quinazoline and Chalcone analogues employing Swiss ADME or Qik Prop of Schrodinger.
- To perform Molecular docking of all the designed series using Auto Dock Tools 4® software
- To synthesize and characterize computationally favorable and some novel analogues of different series using atom efficient processes.
- To perform pharmacological screening of synthesized and characterized analogues on A549 cell line.

STUDY BACK GROUND (LITERATURE REVIEW) AND SIGNIFICANCE OF STUDY

Cancer is due to uncontrolled growth of cells and can be cured if diagnosed at an early stage of life. Treatment of cancer depends on the various internal and external factors. It can be screened by different screening tests and a number of treatments are now available these days, such as Gene therapy, Chemotherapy, Surgery, Radiation therapy, Immunotherapy etc. [1]. Globally, about 9.6 million deaths in 2018 were estimated for cancer, which represents that cancer is the second leading cause of deaths. In India, lung cancer accounts for 5.9% of all cancers and 8.1% of all cancer-related deaths. Increase in adoption of lifestyles with unhealthy

habits like lack of physical activity, decreased intake of fruit and vegetables, increased use of tobacco, fast food, alcohol, etc. [2]. The most common cancers are Lung (2.09 million cases) and Breast (2.09 million cases) [3].

Lung cancer is one of the leading causes of cancer death in the world. If left untreated, this uncontrolled growth can spread beyond the lungs by metastasis into nearby tissue or other parts of the body. The main primary types are small- cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). The vast majority (85%) of cases of lung cancer are due to long-term exposure to tobacco smoke. Other 10–15% of cases are often caused by a combination of genetic factors and exposure to radon gas, asbestos, or other forms of air pollution, including second-hand smoke. [4] According to WHO, within the next two decades, the annual incidence of cancer may increase up to 220 lakhs [2].

The Epidermal Growth Factor Receptor (EGFR) leads to uncontrolled cell proliferation, inhibition of apoptosis, angiogenesis, migration, and metastasis, which are characteristics of cancer cells. EGFR is a first member of the ErbB family of receptor tyrosine kinase. The other members of this family are HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4). EGFR is also known as HER1 or ErbB-1. The over expression of EGFR and HER-2 in human cancer cells such as lung cancer cells made this a potential molecular target for inhibition [5]. The traditional approach to cancer treatment is chemotherapy using cytotoxic drugs which often lack selectivity, high toxicity and subsequently leads to the development of resistance. However, the molecular targeting approach to cancer cells provides increased tumor specificity. EGFR inhibitors have introduced the concept of targeted therapy to the treatment of non-small cell lung cancer (NSCLC). These agents appear most effective in patients with tumors that are highly dependent on EGFR signaling pathways. EGFR inhibition is one of the key targets for cancer chemotherapy. Approval of tyrosine kinase inhibitors such as Erlotinib, Gefitinib, and Lapatinib for the treatment of non-small cell lung cancer has led to tremendous development of novel EGFR inhibitors in the last decade [6]. Currently available EGFR-inhibiting drugs include the tyrosine kinase inhibitors (TKIs) Erlotinib, Gefitinib, and Lapatinib, which are administered orally. The intracellular tyrosine kinase domain, and the Monoclonal antibodies (moAbs or mAbs) Cetuximab and Panitumumab, which are administered intravenously [7].

Heterocyclic nuclei such as quinazoline and indole are constantly being used for the discovery of new lead molecules. Apart from the already marketed drugs, there are many others being investigated for their promising activity against various malignancies. In particular, anticancer research has been capitalizing on the intrinsic versatility and dynamic core scaffold of these compounds [8]. Many of the clinically approved drugs contain the heterocyclic core

including anti-cancer agents such as Osimertinib, Lapatinib, Erlotinib, Gefitinib, etc. Figure 1 [7].

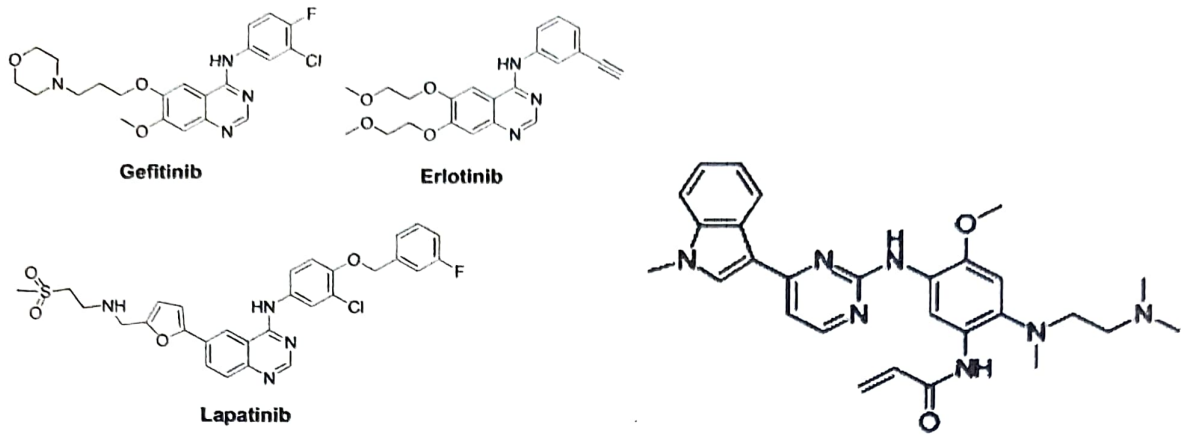


Figure 1. Drugs with Quinazoline and Indole basic core structure [7]

Flavonoids are the largest class of low-molecular-weight polyphenolic secondary metabolites with a benzopyrone nucleus with phenyl ring attached at 2 position (C6-C3-C6) in the molecule. Over 10,000 flavonoids have been described, many of which are present in large quantities in fruits and vegetables [9]. Chalcones (1,3-diaryl-2-propen-1-ones) are precursors for flavonoids and isoflavonoids, which are common simple chemical scaffolds found in many naturally occurring compounds. Chalcones (chalconoids, 1,3-diaryl-2-propen-1-ones) & naphthochalcones and quinazoline shown in (figure 2) are natural and synthetic compounds. Reactive hydrogen from the basic structure of chalcones determines the possibility of these compounds being structurally modified in order to obtain a large number of derivatives. Chalcones analogues have attracted much interest due to their broad biological activities with clinical potentials against various diseases, particularly for antitumor activity. The chalcone family has demonstrated potential *in vitro* and *in vivo* activity against cancers via multiple mechanisms. It represents a promising strategy to develop chalcones as novel anticancer agents. The combination of chalcones and other therapies is expected to be an effective way to improve anticancer therapeutic efficacy [9].

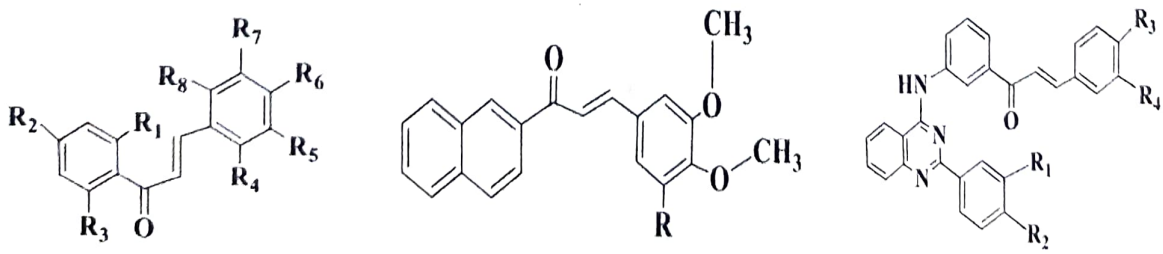


Figure 2: General structure of different chalcones [9]

NATIONAL STATUS

The review on the following researcher's accomplishments gave us ideas about the 1, 3, 4-thiadiazole, indole quinazoline and chalcone analogues scaffold and its analogues which showcase activity in lung cancer and have been tabulated below:

Sr. no	Research Findings	Reference number
1.	Synthesis of substituted imidazo [2,1-b]-1,3,4-thiadiazoles 2a, b-6, substituted 1,3,4-thiadiazolo [3,2-a] pyrimidines 7- 9 and 1,3-disubstituted thioureas 10a-e. All synthesized derivatives were evaluated for their cytotoxic activity against tumor cell line A549 (Non-Small Cell Lung Cancer Cell Line) using Sulfo-Rodamine B (SRB) standard method.	10
2.	Designed and synthesized a new library of amide derivatives of imidazo[2,1-b] [1,3,4] thiadiazoles (11a–j) which were evaluated for their anticancer potentials on A549 (lung carcinoma) by using MTT assay and showed potent anticancer activity.	11
3.	Comprehensive overview of anticancer potentiality of diversely substituted indole derivatives including 1H-indole-2,3- dione and spiro indole derivatives .	12
4.	Summarized the work done in the last five years to overcome the limitations of currently marketed drugs, giving structure activity relationships of quinazoline-based lead compounds synthesized and tested recently. They also highlighted the shortcomings of the currently used approaches and have provided guidance for circumventing these limitations which would help medicinal chemists streamline and guide their efforts towards developing novel quinazoline-based EGFR inhibitors.	13
5.	Some of the chalcones and flavones are very efficient to facilitate the signal of death to the lung cancer cell through blocking the active site BH3 of Bcl-2 proteins. We designed a number of novel chalcones and flavones targeting the active site of the protein. Docking studies showed that the designed compounds were very efficient as the reported compounds.	14

INTERNATIONAL STATUS

The literature review from the other countries provided the following notions:

Sr.no	Research Findings	Reference number
1.	Synthesized and investigated a series of new 1, 3, 4-thiadiazoles for *in vitro* activities against human lung cancer (A-549) cell lines. They compared with cisplatin standard anticancer drug.	15
2.	Studied indole-3-carbinol (I3C) anti-tumor effects on various cancer cell lines. They investigated the anti-tumor effects and its mechanism of I3C on human lung carcinoma A549 cell line.	16
3.	study evaluated the selective effects of 2-isopropyl-3-butyl-8-(4-fluorophenylamino)-3Himidazo[4,5-g] quinazoline (B-2), a member of a series of quinazolines, on the cell survival and growth of the non-small cell lung cancer (NSCLC) cell line A549 *in vitro* and *in vivo*.	17
4.	Presented reports on synthesis and *in vitro* biological characterization of nine pyrazoles derived from chalcones as potential anticancer agents for non-small cell lung cancer A-549.	18

STUDY HYPOTHESES

The overview of literature prompted us to explore heterocycles such as 1,3,4-thiadiazole, indole, quinazoline and chalcones for design of series as potential EGFR inhibitors for non-small cell lung cancer (NSCLC). Computational studies would help us understand in less time and cost-effective way the pharmacokinetics and plausible interactions of designed analogues of different series with EGFR for lung cancer. With this view, *in silico* ADME studies of the designed heterocyclic and chalcone series will be performed. Molecular docking study of these analogues as prospective EGFR inhibitors using pdb id 1M17 with first generation Erlotinib inhibitor and 6LUD with third generation Osimertinib inhibitor will be studied. During the design of various analogues on different scaffolds, some novel analogues would be explored for scope of patent.

The computationally favorable analogues of at least two different series will be synthesized using processes which can be economic and green. The characterization of the synthesized analogues for correctness of their anticipated structures will be ascertained by spectroscopic techniques such as infrared spectroscopy, proton nuclear magnetic resonance spectroscopy and mass spectroscopy. Further, pharmacological screening of the synthesized and characterized analogues will be performed on A549 cell line to test the potential of these proposed EGFR inhibitors on NSCLC.

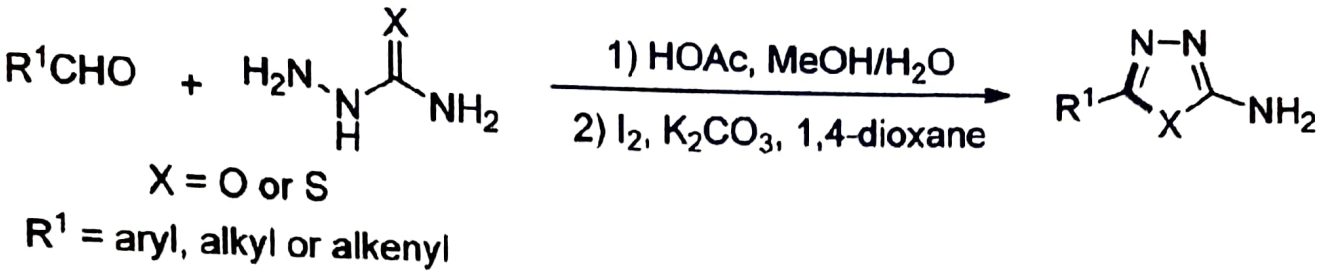
STUDY RATIONALE

The literature survey gave us insight on the scaffolds which can be investigated as EGFR inhibitors for NSCLC. The present research work will be based on design of analogues for series on heterocyclic scaffolds such as 1,3,4-thiadiazole, indole, quinazoline and chalcone. These developed analogues will be computational researched for their drug-like activity using in silico ADME and molecular docking to understand their interactions within the EGFR active site. It will also assist in the selection of suitable analogues for the synthesis and characterization of computationally suitable conventional and novel analogues of at least two different series using atom efficient process with scope for patentability. The synthesized will be subjected to pharmacological testing on the A549 cell line for NSCLC lung cancer.

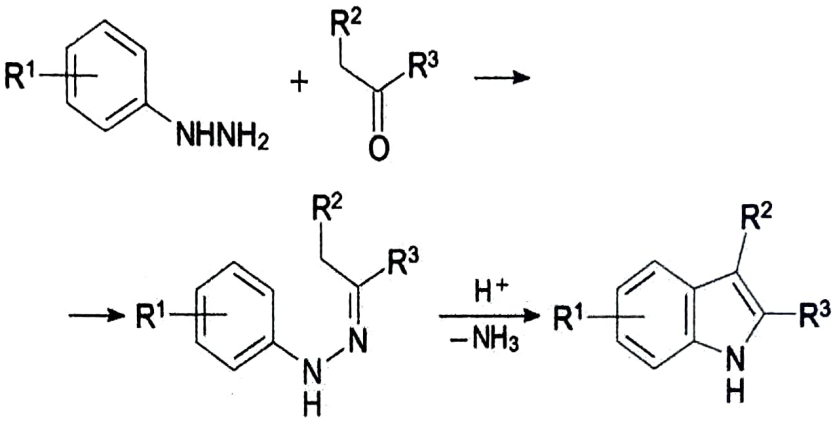
RESEARCH METHODOLOGY

1) Design of ligands based on heterocycles such as 1,3,4-Thiadiazole, Indole, Quinazoline and Chalcone analogues.
2) In *silico* ADME studies of the designed analogues with heterocycle such as 1,3,4-Thiadiazole, Indole, Quinazoline and Chalcone analogues employing Swiss ADME or Qik Prop of Schrodinger.
3) Molecular docking study of these analogues using Auto Dock Tools 4® software, with pdb id Erlotinib 1M17 (first generation) and Osimertinib 6LUD (third generation), the marketed drugs with good inhibitory activity profile.
4) Using an atom-efficient technique, some unique and computationally advantageous analogues of various series will be synthesized and characterized. (40 compounds). Following schemes will be explored for synthesis of 1, 3, 4-thiadiazole, Indole, Quinazoline and chalcones analogues with monitoring of reaction by TLC (Schemes: 1,2,3,4).

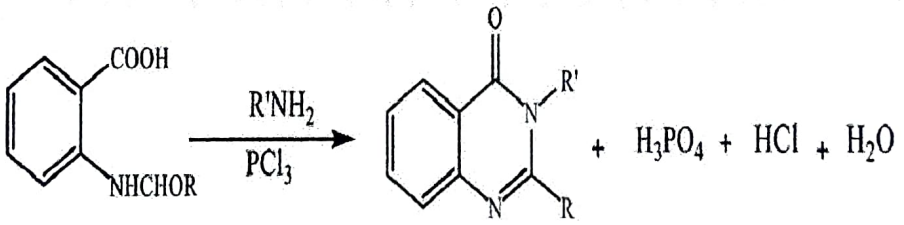
Scheme 1: General reaction for synthesis of 1, 3, 4-thiadiazole [19]



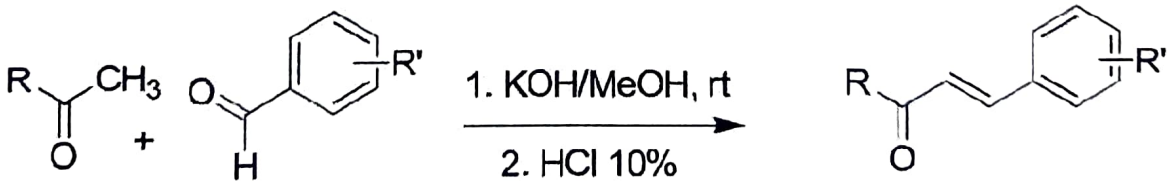
Scheme 2: General reaction for synthesis of Indole [20]



Scheme 3: General reaction for synthesis of Quinazoline [21]



Scheme 4: General reaction for synthesis of Chalcone [22]



5) Characterization of the synthesized compounds will be accomplished by Infrared Spectroscopy, ^{1}H Nuclear Magnetic Resonance Spectroscopy.

6) Pharmacological screening of synthesized and characterized analogues will be performed on A549 Cell line.

ACTIVITY CHART

Sr.no	Activity	1 – 6 Months	6 – 12 Months	13 – 18 Months	18 – 24 Months	24 – 30 Months	30 – 36 Months
1	Literature Search	√	√	√	√	√	√
2	Computer-aided drug design	√	√		√	√	
3	Molecular docking	√	√		√	√	
4	*In silico* ADME studies	√	√		√	√	
5	Synthesis of molecules		√	√	√	√	
6	Characterization of the synthesized compounds			√	√	√	√
7	Pharmacological evaluation						√
8	Publication		1		2		3

LIMITATIONS OF THE STUDY:

The detailed enzyme kinetic studies and biochemical assays for the promising analogues which need extensive work and expertise can be explored as part of next project.

EXPECTED OUTCOMES OF STUDY

Design of potential EGFR inhibitors series based on heterocyclic scaffolds such as 1,3,4-thiadiazole, indole, quinazoline, and chalcones for NSCLC lung cancer, with successful computational outcomes like Molecular docking and *in vivo* pharmacokinetics. Further, synthesis and characterization of computationally favorable and some novel analogues of different explored series using atom-efficient process. The possibility of patentability for the novel analogues and process. Evaluating the pharmacological profiles of the synthesized analogues on the A549 cell lines.

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